

We claim:

1. A method for determining a presence of bacteria in a sample containing platelets comprising the steps of:

lysing a substantial portion of the platelets in the sample;

5 staining the bacteria using a membrane-permeable nucleic acid stain;

filtering the sample using a membrane filter to retain a material

containing stained bacteria on the filter; and

analyzing the material using epifluorescence microscopy to determine the presence of bacteria in the sample.

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2. A method as claimed in claim 1, wherein the step of lysing the platelets is carried out by contacting the platelets with a suitable amount of a suitable lytic agent for a sufficient period of time to lyse 90% of the platelets.

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3. A method as claimed in claim 1, wherein the step of lysing the platelets is carried out by contacting the platelets with a suitable amount of a suitable lytic agent for a sufficient period of time to lyse 99% of the platelets while destroying less than 20% of the bacteria in the sample.

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4. A method as claimed in claim 3, wherein the suitable amount of the lytic agent ranges from about 0.5% to about 20% of the platelets by volume, and the lytic agent is a detergent.

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5. A method as claimed in claim 1, wherein the step of analyzing the material further comprises the steps of:

acquiring digital images of the material;

analyzing the digital images to determine a count of the bacteria; and

comparing the count of the bacteria with a threshold count to ascertain the presence of bacteria.

6. A method as claimed in claim 1, wherein the membrane-permeable nucleic acid stain comprises a SYTO dye.
7. A method as claimed in claim 1, wherein the membrane-permeable nucleic acid stain is SYTO 13 and the lytic agent is Triton X-100.
8. A method as claimed in claim 1, wherein the step of staining the bacteria comprises the step of contacting the sample with the membrane-permeable nucleic acid stain for about 1 to about 15 minutes.
9. A method as claimed in claim 1, wherein the membrane filter has a pore size between about 0.2  $\mu\text{m}$  and about a diameter of a bacteria cell.
10. A method as claimed in claim 1, wherein a material containing substantially all of the stained bacteria is retained on the membrane filter after the filtering step, and wherein the method further comprises the step of drying the material retained on the membrane filter after the sample has been filtered through the membrane filter.
11. A method as claimed in claim 1, wherein the steps of lysing a substantial portion of the platelets in the sample and staining the bacteria using a membrane-permeable nucleic acid stain are carried out simultaneously.
12. A method for determining a concentration of bacteria in a sample containing platelets, comprising the steps of:
- lysing a substantial portion of the platelets without destroying a substantial amount of bacterial cells in the sample;
  - staining the bacteria using a membrane-permeable nucleic acid stain;
  - filtering the sample using a membrane filter to retain a material

containing substantially all of the stained bacteria on the filter; and  
analyzing the material using epifluorescence microscopy and digital  
image acquisition and analysis to determine the concentration of the bacteria in  
the sample.

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13. A method claimed as in claim 12, wherein the step of analyzing the material  
further comprises the steps of:

acquiring digital images of the material;

analyzing the digital images to determine a count of the bacteria; and

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comparing the count of the bacteria with a calibration curve to determine  
the concentration of the bacteria.

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14. A method as claimed in claim 12, wherein a material containing  
substantially all of the stained bacteria is retained on the membrane filter after  
the filtering step, and wherein the method further comprises the step of drying  
the material retained on the membrane filter after the sample is filtered through  
the membrane filter.

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15. A method as claimed in claim 12, wherein steps of lysing a substantial  
portion of the platelets without destroying a substantial amount of bacterial cells  
in the sample and staining the bacteria using a membrane-permeable nucleic  
acid stain are carried out simultaneously.

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16. A method for ascertaining a presence of bacteria in a platelet suspension  
having platelets comprising the steps of:

lysing a substantial portion of the platelets without destroying a  
substantial amount of the bacteria in the platelet suspension;

staining the bacteria using a membrane-permeable nucleic acid stain;

filtering the platelet suspension using a membrane filter with a suitable

pore size, to retain a material containing substantially all of the stained bacteria on the filter; and

analyzing the material using epifluorescence microscopy and digital image acquisition and analysis to determine the presence of the bacteria in the platelet suspension.

17. A method as claimed in claim 16, wherein the step of lysing the platelets is carried out by contacting the platelet suspension with a sufficient amount of a lytic agent

18. A method as claimed in claim 17, wherein the amount of the lytic agent ranges from about 0.5% to about 20% of the platelet suspension.

19. A method as claimed in claim 17, wherein the lytic agent is a detergent.

20. A method as claimed in claim 17, wherein the membrane-permeable nucleic acid stain is SYTO 13 and the lytic agent is Triton X-100.

21. A method as claimed in claim 16, wherein the step of analyzing the material further comprises the steps of:

acquiring digital images of the material using an automated epifluorescence microscope;

analyzing the digital images using an image analysis program to determine a count of the bacteria; and

comparing the count of the bacteria with a threshold count to determine the presence of the bacteria in the platelet suspension.

22. A method as claimed in claim 16 further comprising the step of drying the material retained on the membrane filter after the platelet suspension has been

filtered through the membrane filter.

23. A method as claimed in claim 16 wherein steps of lysing a substantial portion of the platelets without destroying a substantial amount of the bacteria  
5 in the platelet suspension and staining the bacteria using a membrane-permeable nucleic acid stain are carried out simultaneously.

24. A method for determining a presence of bacteria in a sample containing red blood cells comprising the steps of:
- 10 lysing a substantial portion of the red blood cells in the sample;  
staining the bacteria using a membrane permeable nucleic acid stain;  
filtering the sample using a membrane filter to retain a material  
containing stained bacteria on the filter; and  
analyzing the material using epifluorescence microscopy to determine  
15 the presence of bacteria in the sample.

25. A method as claimed in claim 24, wherein the step of lysing the red blood cells is carried out by contacting the red blood cells with a suitable amount of a suitable lytic agent for a sufficient period of time to lyse at least 90% of the red  
20 blood cells.

26. A method as claimed in claim 24, wherein the step of lysing the red blood cells is carried out by contacting the red blood cells with a suitable amount of a suitable lytic agent for a sufficient period of time to lyse at least 99% of the red  
25 blood cells while destroying less than 20% of the bacteria in the sample.

27. A method as claimed in claim 24, wherein the step of analyzing the material further comprises the steps of:

acquiring digital images of the material;

analyzing the digital images to determine a count of the bacteria; and  
comparing the count of the bacteria with a threshold count to ascertain  
the presence of bacteria.

5    28. A method as claimed in claim 24, wherein the membrane-permeable  
nucleic acid stain is SYTO 13 and the lytic agent is Triton X-100.

29. A method as claimed in claim 24, wherein steps of lysing a substantial  
portion of the red blood cells in the sample and staining the bacteria using a  
10    membrane permeable nucleic acid stain are carried out simultaneously.